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OUTLINE FOR THE INVESTIGATION OF
A FUNGUS STAIN ASSOCIATED WITH
SCOLYTUS VENTRALIS LEC. GALLERIES.

By Ernest Wright,
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In a preliminary report for the season 1930 entitled, "The fir engraver beetle and associated insects in white fir", Geo. R. Struble, Assistant Forest Entomologist, gives an account of the isolation of a stain fungus found in constant association with Scolytus ventralis Lec. galleries. Inoculations of white firs with the isolated fungus produced the identical stain as found in association with the beetle attacks. The Division of Forest Pathology has been asked to cooperate with Forest Entomology for the season 1931 for the purpose of continuing the study of this stain organism.

It has been decided that the primary object of the current season's investigation should be to determine the relationship of the stain to beetle development, if such a determination is possible. Other objects introduced are important but actually are contributory to the main object.

Below is given the proposed outline and it is hoped that those interested will feel free to make suggestions and criticisms.

I. Object: To establish definitely in what percentage of the cases the fungus stain is present when succesful beetle attacks occur.

Procedure:

1. In cooperation with Entomology examine trap logs and fading white fir trees where S. ventralis broods are present.
 - (a) Count and measure S. v. galleries in millimeters.
 - (b) Count galleries where stain is present and trace off stained areas. Note: From these tracings the area of stain can later be computed by planimeter.
 - (c) Compute area of each bark section studied. Measure bark thickness.
2. In cooperation with Entomology use sections of bark which show stages of attacks one week, two weeks, three weeks etc. old. Follow the procedure as outlined above, sketching the area stained. Note: This will give the approximate spread of stain at weekly intervals.
3. Examine felled Entomology caged trees where beetle attacks have been forced and examine stained area in
 - (a) Successful attacks.
 - (b) Unsuccessful attacks.

II. Object: To isolate organism from fresh material taken from numerous sources. Isolations for inoculation and comparative purposes.

Procedure:

1. Culture the organism from stained areas associated with numerous S. v. galleries. As a medium use malt agar in test tube slants.
 - (a) As soon as mycelial growth develops sufficiently transfer to fresh agar slants.
 - (b) Compare transfers microscopically with transfers of Struble's original cultures.
 - (c) To determine penetration of stain make cultures from various depths both in the inner bark and sapwood.
 - (d) To determine extent of stain make cultures at intervals beyond visible stain in both inner bark and sapwood.
 - (e) Make cultures from inner bark and sapwood of old attacks to determine viability of stain.
 - (f) Determine position of mycelium in recent attacks whether inter or intracellular by preparing sections and examining microscopically.

III. Object: To further substantiate Struble's findings that the stain can be artificial introduced into green white firs and will spread without the assistance of beetles.

Procedure:

1. Using transfers from II 1(a) after growth is well developed make 200 inoculations in small to medium sized standing white firs. Alternate with 200 sterile agar checks in the same trees. Note: It is planned to use a 3/32 inch drill for making inoculation holes and to make the same type of inoculation as Struble did in 1930, but use 95 per cent alcohol as a sterilizing agency also plug inoculation holes in 50% of the cases with sterile cotton. It is possible to alternate the checks with the stain inoculations on the same trees as the organism only kills the cambium locally. By using the same trees for both checks and inoculations, conditions will be as comparable as possible. It is planned to make the inoculations spirally around the tree trunks and all trees will be located on the same site. A total of not over 10 trees will be used.

(a) Examine a few inoculations at weekly intervals and determine rate of spread. Also examine the same number of checks on the same trees.

(b) Re-isolate the stain organism. Compare with original cultures. With re-isolated cultures inoculate several other trees and examine periodically. Or in other words follow Koch's rules.

IV. Object: To determine the influence of stain on successful beetle attacks.

1. On two trees heavily inoculated with 60 to 100 inoculations, including checks, and with the cooperation of Entomology, cage after 3 weeks and introduce S. ventralis beetles. Note: The purpose of this is to see whether the beetles in attacking (if they do attack) will select only the stained areas in which to work.

V. Object: To determine how stain may be carried by beetles and the relationship existing between the beetles and the stain.

Procedure:

1. Collect beetles just attacking, just emerging and already established in tree or trap logs. (Secure from Ent.)

- (a) Allow beetles collected as above to walk over poured sterile agar plates.
- (1) Isolate organisms which become established and compare with stain cultures.
2. Dissect beetles collected as above, sterilize parts and plate in sterile agar.
- (a) Isolate organisms which become established and compare.
3. Crush pupae and place in sterile agar plates.
- Note: This may indicate whether the stain spores are carried through the pupal stage depending upon the success obtained also with adult beetles.
4. On agar plates where stain cultures are growing introduce larvae and observe if feeding takes place.
5. In case one or two organisms are constantly isolated from beetle cultures try tree inoculation and examine periodically for stain.

Discussion of Problem.

It is felt that the factors influencing the development of the stain such as temperature, bark thickness, light, aëration and moisture content, should not be included in this season's study. It has been indicated by Struble and so far substantiated by myself that stain is present in practically 100% of successful beetle attacks and individual factors influencing the development of stain can be studied at a later date if advisable.

Identification of the organism will necessarily be confined to further laboratory work and to outside assistance.

Introducing beetles into sterile logs under sterile conditions is part of the program planned for this winter's work in the Berkeley lab. by Struble and I will cooperate in such parts as my assistance will be required.